

Ecophysiological responses and carbon distribution of *Pinus koraiensis* seedlings to elevated carbon dioxide

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Abstract: The net CO₂ assimilation rate, stomatal conductance, RuBPCase (ribulose 1,5-biphosphate carboxylose) activity, dry weight of aboveground and belowground part, plant height, the length and diameter of taproot of *Pinus koraiensis* seedlings were measured and analyzed after six-week exposure to elevated CO₂ in an open-top chamber in Changbai Mountain of China from May to Oct. 1999. Seedlings were planted in four different conditions: on an open site, control chamber, 500 $\mu\text{L}\cdot\text{L}^{-1}$ and 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ chambers. The results showed that the total biomass of the seedlings increased whereas stomatal conductance decreased. The physiological responses and growth to 500 $\mu\text{L}\cdot\text{L}^{-1}$ and 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ varied greatly. The acclimation of photosynthesis was downward to 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ but upward to 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂. The RuBPCase activity, chlorophyll and soluble sugar contents of the seedlings grown at 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ were higher than that at 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂. The concentration 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ enhanced the growth of aboveground part whereas 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ allocated more carbon to belowground part. Elevated CO₂ changed the carbon distribution pattern. The ecophysiological responses were significantly different between plants grown under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ and 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂.

Key words: Net CO₂ assimilation; Stomatal conductance; RuBPCase; *Pinus koraiensis*; Biomass

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Introduction

The concentration of atmospheric CO₂ has increased since the industrial revolution and is predicted to increase in the future (Houghton *et al.* 1992). Carbon dioxide is the first link from atmosphere to biosphere (Hugo & Brett, 1994). Forests cover about 40% of the world's terrestrial surface and are estimated to contain about 80% of terrestrial aboveground carbon and 40% of carbon belowground (Jon *et al.* 1996). The role of forests in the global carbon cycle has been emphasized (Tans *et al.* 1990). Trees constitute a major reservoir of carbon (Tans *et al.* 1990) by assimilation and fixation of CO₂. The ecophysiological reaction varied greatly with time and species to elevated CO₂, especially in C₃ plant. There hasn't been a comprehensive theory on plant response to elevated CO₂ by now.

CO₂ and water are combined in plant leaves utilizing sunlight to produce carbohydrates and oxygen

(Kimball *et al.* 1993). Other biological substances forming the plant body such as proteins and lipids are made from carbohydrate. Photosynthesis sustains life basis so that there is interest in determining the response of plants to increased atmospheric CO₂ concentration. There are different study results in plants exposed to elevated CO₂ between short time and long-time on which there isn't definite boundary. The net CO₂ assimilation rate in C₃ plants exposed to long-term high CO₂ doesn't always increase. In some cases, it is unchanged or may indeed decrease in comparison with that in plants grown at ambient CO₂ concentration (Sage *et al.* 1989; Jarvis 1993). Stomatal conductance was significantly decreased in leaves grown in CO₂-enriched air on most measurement data. This has been observed previously in many studies (Eamus & Jarvis 1989; Sage 1994). Chlorophyll content shared the same trend. The net CO₂ assimilation versus intercellular CO₂ concentration response curve has been widely used (Sage *et al.* 1989; Jarvis 1993) to explain RuBPCase capacity with its initial slope. In this study, the enzyme activity, net CO₂ assimilation and growth state were measured in parallel so that they can be compared with each other accurately. The direct product of photosynthesis is carbohydrate. The change of soluble sugar content is essential to account for the plant response to different CO₂ concentration. In some studies, CO₂ enrichment has been found to have little or no effect on the con-

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centration of soluble sugar of leaves (Mauney *et al.* 1979). But in recent studies, soluble sugars were reportedly increased with CO₂-enriched.

Photosynthesis, biomass accumulation and economic yield are nearly always stimulated by elevation of CO₂ concentration (Hugo *et al.* 1994). Analyses of plant responses to elevated concentration of atmospheric CO₂ have focused largely on processes occurring aboveground (Richard 1994). With the elevated CO₂, the question of the "missing carbon" within the global carbon cycle has been put forward (Post *et al.* 1990; Siegenthaler & Sarmiento 1993; Tan *et al.* 1990). Freeman Dyson thought there was connection between the "missing carbon" and root (Dyson 1992). Thus, the study on root systems is very important to thoroughly interpret the effects on the whole plant individual. As a part of plant, root needs nutrient acquisition provided by photosynthesis. At the same time, root is an important organ of carbon storage (Richard 1994). The primary root response variables to elevated CO₂ were root dry weight, root to total shoot ratio, root length and root number (Hugo 1994). Other aspects of plant roots such as structure, function and rhizosphere were also reported. That aboveground and belowground are integrated to interpret the plant response to CO₂-enriched is very important. Aboveground part alone is not enough to specify the interaction between plant individual and changing atmosphere CO₂ concentration so that the studies on root systems are significant component of the analysis.

The studied plants were situated at the Open Research Station of Changbai Mountain Forest Ecosystems, Chinese Academy of Sciences. Korean pine (*Pinus koraiensis*) seedlings were planted in the ground in open-top chambers and on the open site. The experimental environment avoided the shortage of nutrition and water stress resulted from limitation of pot microenvironment previously. In this study, aboveground physiological reactions were integrated with belowground processes from enzyme activity to biomass in order to comprehensively analyze.

Methods

Experimental conditions

In June 1999, Korean pine seedlings were grown from seed sown in soil in open-top chambers (OTC) and on an open site. The seedlings were treated with four different environments during the period of experiment, on an open site, control chamber, 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ and 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ chambers. Every chamber with alloy framework is 1.2 m in length, 0.9 m in width and 0.9 m in height. Glass 3mm thick covered the chamber whose top is open. Elevated CO₂ concentration in OTC were controlled and con-

tinuously monitored in the following way: The teflon pipe with 2 cm inside diameters was fixed in the bottom around the chamber. There are many small holes on pipes through which CO₂-enriched air was injected in the chambers. The pipes were connected with bag outside the chamber. CO₂ in the bag was mixture of pure industrial CO₂ and atmosphere that was pumped into the OTC by the pump. CO₂ concentration was maintained at the steady level by controlling the amount and the flow rate of mixed gas in the bag. Two chambers received high CO₂-enriched air (700 and 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂) and plants in the control chamber and on an open site received ambient air (about 350 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂). In our study, elevated CO₂ was provided day and night since June. After six weeks treatment, measurements of RuBPCase activity, net CO₂ uptake rate, stomatal conductance, chlorophyll and soluble sugar contents were made on Korean pine seedlings in parallel. The sampling time is during at 9:00 a.m. and 10:00 a.m. for clear sunny day when is consistent with measurement of CO₂ uptake.

Measurements of net CO₂ assimilation and stomatal conductance

The rate of net CO₂ assimilation and stomatal conductance were measured with a CI-301 portable infrared gas analyzer in an open system. A series of data were collected automatically and simultaneously every half a minute. Net photosynthetic rate and stomatal conductance of Korean pine seedlings in different treatments were determined every two hours from 6:00 a.m. to 6:00 p.m. so that we can make their daily course.

RuBPCase activity assay

One gram fresh weight of leaves tissue of seedlings from each treatment was grounded in an ice-chilled mortar containing 5 mL (pH 7.8) Tris-HCl 100 mmol/L, EDTA 2 mmol/L, 2% PVP. After centrifugation at 11000 \times g at 4°C for 10 min, supernatant was collected in test tubes and stored at -80°C. 0.3 mL RuBPCase was mixed with 0.3 mL of 0.1M Tris-HCl (pH 7.8), 0.2M MgCl₂, 50 mM DTT, 1mM NADH, 1 mM EDTA and 0.1 mL of 0.5M KHCO₃. The mixture was incubated for 20 min at 40°C to activate RuBPCase. Adding to 0.1 mL PGK and GAPDH, the initial value of optical density of the mixture at 340 nm wavelength was written down. The reaction started by adding 0.1 mL of 0.5 mM RuBP. According to the change of the value of optical density at 340 nm wavelength, the RuBPCase activity was measured.

Contents of soluble sugar and chlorophyll (chl)

Chlorophyll (chl_a, chl_b, total chl) of fresh leaves was extracted in 80% acetone solution and the optical density of the extracts were measured at 645 and

663 nm wavelength against an acetone blank. Chlorophyll content was calculated according to the following equation (Zhang 1992):

$$\text{Chla} = [12.7(D_{663}) - 2.69(D_{645})] \times 0.1 \text{ mg/g}$$

$$\text{Chlb} = [22.9(D_{645}) - 4.68(D_{663})] \times 0.1 \text{ mg/g}$$

$$\text{Total chl} = [20.2(D_{645}) + 8.02(D_{663})] \times 0.1 \text{ mg/g}$$

The fresh leaves were dried to constant weight at 80°C. Soluble sugars (total soluble sugar, sucrose, fructose) of dry leaves were extracted in 80% alcohol at 80°C for 30 min for three times. Measurement of sucrose, fructose and total sugar was controlled at different conditions. Measurement of the sucrose: 0.1 mL soluble sugars extracts were mixed with 0.1 mL 30% KOH at boiling water for 10 min, then mixed with 3 mL anthrone solution at 40°C for 10 min and the optical density of mixture were measured at 620 nm wavelength;

Measurement of the fructose: 0.1 mL soluble sugars extracts were mixed with 3 mL anthrone solution and placed at about 20°C for 90 min, then the optical density of mixture were measured at 620 nm wavelength; Measurement of total sugar: the mixture of extracts and anthrone was at 90°C for 15 min, then the optical density of mixture were measured at 620 nm wavelength (Shanghai Institute of Plant Physiology 1985).

Growth analysis

Plants from each treatment were selected at random and plant height aboveground, length and diameter of taproot, biomass of aboveground and belowground were measured. The entire aboveground and belowground part of the seedlings was dried to constant weight at 80°C. Prior to drying, the plant height aboveground, the length and diameter of taproot were determined with vernier calipers.

Results

Net CO₂ assimilation rate and stomatal conductance

Korean pine seedlings in four treatments showed different trends in daily course of net CO₂ assimilation rate and stomatal conductance. The seedlings grown at 700 and 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ had a single peak photosynthesis curve and those grown at ambient air (in the control chamber and on the open site) had a double peaks photosynthesis curve (Fig.1). The maximum of net photosynthetic rate under 700 and 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ appeared at 8:00 a.m. Then CO₂ assimilation gradually declined. In contrast, the maximum of net photosynthetic rate also appeared at 8 a.m. in ambient CO₂, but fell to a minimum at 12:00. After 12:00, net CO₂ assimilation rate increased again. It didn't decrease until 14:00 p.m.

Under high CO₂ concentration, the decrease or

increase in net CO₂ assimilation was accompanied by decrease or increase in stomatal conductance. When stomatal conductance decreases, resistance of CO₂ coming into the cell increases as will affect the inter-cellular CO₂ concentration. However, in the control chamber and on the open site the relationship between net CO₂ assimilation and stomatal conductance wasn't the same as that under elevated CO₂. For instance, at 12:00, net CO₂ assimilation decreased to the minimum while stomatal conductance had a steep rise (Fig.2). The phenomenon that the decline in net CO₂ assimilation was associated with increased stomatal conductance was also found in previous studies. So the reduction of stomatal conductance wasn't sufficiently large to account for the observed decline in photosynthesis (Evan *et al.* 1985).

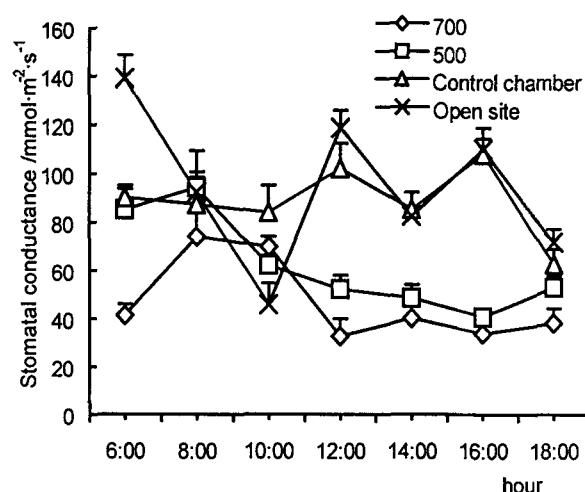


Fig.1. Daily course of net CO₂ assimilation of *Pinus koraiensis* seedlings under 700 $\mu\text{L}\cdot\text{L}^{-1}$, 500 $\mu\text{L}\cdot\text{L}^{-1}$ and ambient CO₂ concentration

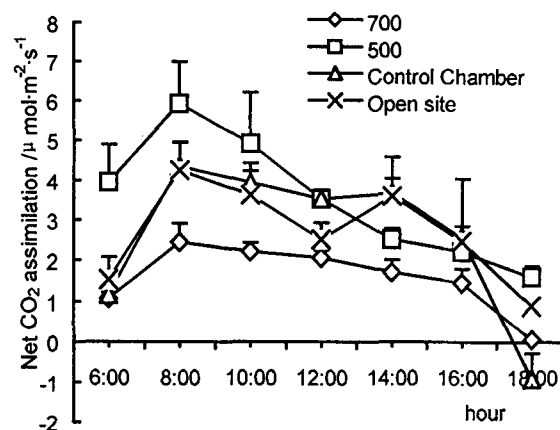


Fig.2. Daily course of stomatal conductance of *Pinus koraiensis* seedlings under 700 $\mu\text{L}\cdot\text{L}^{-1}$, 500 $\mu\text{L}\cdot\text{L}^{-1}$ and ambient CO₂ concentration

According to the average values of daily net CO₂ assimilation and stomatal conductance based on the many instantaneous measurements (Table 1), we draw the following conclusions: Net CO₂ assimilation under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ was the highest, then on the open site and in the control chamber. Under 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ the net photosynthetic rate was the least. Stomatal conductance at ambient CO₂ concentration was significantly large compared with that under the elevated CO₂. Especially, stomatal conductance and

net CO₂ assimilation under 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ was the smallest. It is suggested that 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ can increase net CO₂ assimilation of Korean pine seedlings. Although stomatal conductance under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ was reduced compared with that at ambient CO₂, intercellular CO₂ concentration remained enhanced that can make compensation for decline of the stomatal conductance.

Table 1. The average daily net CO₂ assimilation and stomatal conductance of Korean pine seedlings grown at different CO₂ concentrations

	700 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂	500 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂	Control chamber (350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂)	Open site (350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂)
Net CO ₂ assimilation ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (n=7)	1.57 \pm 0.82	3.51 \pm 1.54	2.60 \pm 1.16	2.68 \pm 1.21
Stomatal conductance ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (n=7)	47.3 \pm 17.32	62.5 \pm 19.99	88.70 \pm 14.51	94.44 \pm 31.24

Net CO₂ assimilation and stomatal conductance alone are not enough to generalize the nature of plant responses to elevated CO₂ concentration. They should be integrated with other ecophysiological data to gain more detailed analysis. The photosynthetic responses in Korean pine seedlings varied greatly owing to their acclimation to different CO₂ concentration. Net CO₂ assimilation under elevated CO₂ concentration can't be simply interpreted as "downward" or "upward" regulation according to our results. In previous studies, different species responded differently to various level CO₂ concentrations according to long-term or short-term treatment. By now, there hasn't a common view or understanding on the affection of high CO₂ concentration.

Ecophysiological indexes

RuBPcase under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ activity showed a marked increase, so did the contents of soluble sugar and chlorophyll. These physiological indexes increased in parallel with net CO₂ assimilation. But the Chl a/b ratio showed the opposite trend (Table 2). Plants grown under 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ had the lowest not only photosynthetic capacity but also the contents of soluble sugar and chlorophyll and RuBPcase activity. Especially, the chlorophyll contents are obviously low. During the growth, we found two albinos. We don't dare say 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ may cause this phenomenon, but at least there is some relationship between albino and low chlorophyll contents. However, 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ caused an increase in Chl a/b ratio. Korean pine is a C₃ plant and CO₂ concentration is the main limitation to its photosynthesis. The current concentration of CO₂ in air restricts photosynthesis of most plants (Lemon 1983). 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ can increase the intercellular CO₂ concentration of

Korean pine seedlings. RuBPcase is the initial CO₂-fixation enzyme which controls net CO₂ assimilation (Park *et al.* 1998). CO₂ concentration at the catalytic site limits the activity of RuBPcase (Stitt 1991). The higher intercellular CO₂ concentration is essential to improve photosynthetic capacity since both CO₂ and O₂ compete for the same site on the catalyzing enzyme RuBPcase (Hugo *et al.* 1994). High CO₂ reduces competition from O₂ for RuBPcase and increase its carboxylase activity. 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ level stimulate photosynthesis thus produce more soluble sugars in the leaves.

The results of physiological measurements are consistent with the growth for Korean pine seedlings grown in four different conditions. Under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ concentration, the aboveground dry weight and plant height are the greatest among the four conditions which is parallel with net CO₂ assimilation and soluble sugar contents. Belowground dry weight, the length and diameter of the taproot had the obvious predominance under 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ which was opposite to net CO₂ assimilation and soluble sugar contents of leaves.

CO₂ enrichment (including 500 $\mu\text{L}\cdot\text{L}^{-1}$ and 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂) caused increase in total biomass compared with that in the ambient air. The root dry weight, at 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂, increased by 9.0% and 44.8%, taproot length by 9.3% and 9.0%, taproot diameter by 2.9% and 5.8% compared with control and open site respectively. Plant height and dry weight of aboveground part of plants grown at 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ increased by 10.2% and 12.5% compared with those in the control chamber, and increased by 2.4% and 35.8% compared with those on the open site. But the decrease of length and diameter of taproot under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ was very apparent though aboveground

part had positively physiological reaction (Table 3).

Table 2. Measurements of RuBPCase activity and substance contents at different CO₂ concentrations

Item	700 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂	500 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂	Control chamber (350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂)	Open site (350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂)
RuBPCase ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mgPr}^{-1}$)	0.410	0.534	0.447	0.472
Chlorophyll a (n=4)	0.127 \pm 0.008	0.264 \pm 0.04	0.236 \pm 0.02	0.256 \pm 0.02
Chlorophyll b (n=4)	0.081 \pm 0.004	0.173 \pm 0.04	0.155 \pm 0.01	0.165 \pm 0.03
Total Chlorophyll (n=4) (mg \cdot g ⁻¹ fresh leaves weight)	0.208 \pm 0.010	0.436 \pm 0.08	0.391 \pm 0.03	0.421 \pm 0.04
chl a/b	1.586	1.526	1.523	1.552
Sucrose (n=3)	11.33 \pm 1.27	12.87 \pm 1.13	10.13 \pm 0.26	12.40 \pm 2.32
Fructose (n=3)	8.79 \pm 0.83	8.79 \pm 1.06	8.85 \pm 0.89	6.47 \pm 2.41
Total soluble sugar (n=3) (mg \cdot g ⁻¹ dry leaves weight)	29.63 \pm 3.41	32.56 \pm 3.87	30.38 \pm 2.67	30.44 \pm 4.71

Table 3. Measurements of aboveground and belowground biomass, plant height, length and diameter of taproot for Korean pine seedlings grown at different CO₂ concentrations

Item	700 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂	500 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂	Control chamber (350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂)	Open site (350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂)
Aboveground dry weight (g) (mean \pm SD)	0.448 \pm 0.026(4)	0.480 \pm 0.090(3)	0.420 \pm 0.065(5)	0.308 \pm 0.056(4)
Belowground dry weight (g) (mean \pm SD)	0.145 \pm 0.012(3)	0.118 \pm 0.011(3)	0.132 \pm 0.011(3)	0.080 \pm 0.016(3)
Total dry weight (g)	0.593	0.598	0.552	0.388
Ratio of root to shoot	0.324	0.246	0.314	0.260
Plant height (cm)	6.20 \pm 0.666(4)	6.35 \pm 0.238(4)	5.70 \pm 0(3)	6.20 \pm 0.424(5)
Taproot length (cm)	15.25 \pm 1.666(4)	11.55 \pm 0.238(4)	13.83 \pm 1.286(3)	13.88 \pm 0.613(4)
Taproot diameter (cm)	0.173 \pm 0.005(4)	0.160 \pm 0(4)	0.168 \pm 0.003(3)	0.163 \pm 0.004(5)

Under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ Korean pine seedlings had higher rate of net photosynthesis and soluble sugar contents than those under 700 $\mu\text{L}\cdot\text{L}^{-1}$ or ambient CO₂. More photosynthates were distributed to aboveground part to produce new leaves and stretch stem. Vigorous growth of aboveground resulted in a restricted effect on taproot that made root growth evidently slower than control and open site. 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ concentration can stimulate translocation of carbohydrates produced in leaves, namely 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ treatment had a profound effect on distribution pattern of carbohydrate. Root-to-shoot ratio and total dry weight indicated more carbon were allocated in aboveground part under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ whereas more carbon were assigned to belowground part under 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂.

Discussion

The net CO₂ assimilation and stomatal conductance of Korean pine seedlings responded differently to elevated CO₂ and ambient CO₂ concentrations. For plants in the control chamber and on the open site, the decreased photosynthesis rate at noon was associated with the increase in stomatal conductance,

which indicated that there were non-stomatal limitations to photosynthesis. In other words, there were other factors besides stomata affecting the CO₂ assimilation rate. The decline in net CO₂ assimilation despite enhanced stomatal conductance was also reported by Eamus *et al* (1993). It is possible that the decline of net CO₂ assimilation was caused by RuBPCase. At noon, high temperature results in decline in the RuBPCase activity. RuBPCase limits net CO₂ assimilation (Park *et al.* 1998). In addition, respiration increase caused by high temperature at noon resulted in apparent decline of net CO₂ assimilation.

Every ecophysiological index of Korean pine seedlings grown under 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ was obviously lower than that under 500 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ and ambient CO₂. The reasons may be as following: The initial high level of photosynthesis that occurred when the seedlings were first exposed to 700 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂ levels may decrease after a temporal period (Hugo 1994). There are numerous intermediate steps before the carbohydrates produced in the leaves are transformed into root or additional leaf tissue (Kimball *et al.* 1993). Because Korean pine seedlings' root system, young tissues couldn't absorb, transform and utilize thoroughly all those photosynthesis products, non-structural carbohydrate was accumulated in the

leaves. It is suggested the sink demand cannot accommodate to the enhanced level of photosynthate when Korean pine seedlings were exposed to $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 for a period. The equilibrium between source and sink regulation disrupted. Carbohydrate accumulation in the leaves caused partial closure of stomata, which would restrict CO_2 diffusing. As a result, the net CO_2 assimilation and soluble sugar contents of the leaves decreased with the decline of CO_2 fixation. Maybe there was another reason of RuBPCase. $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 inhibited carbon assimilation by reducing RuBPCase activity. From the experimental results, we know the RuBPCase activity under $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 was the lowest. Eamus *et al.* suggested that a depletion of phosphate supply to the chloroplast could result in a lower A_{max} . Phosphate requirement is increased by CO_2 enrichment as a result of an increase in A_{max} (Conroy 1992). There are many other possible mechanisms of feedback inhibition on photosynthesis including biochemical regulation of key anabolic and catabolic enzymes. It is reasonable to expect that several different mechanisms can operate simultaneously (Evan *et al.* 1985).

In this study, CO_2 enrichment under $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 produced a decline in net photosynthesis, contents of chlorophyll and soluble sugar and RuBPCase activity. Whereas plants grown in $500 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 concentration had higher net CO_2 assimilation and soluble sugar contents, which suggested that the photosynthesis was upward regulation and had an active sink to ensure the transportation of photosynthates from the chloroplasts.

There have been so many studies examining the effects of CO_2 on plants, of which a relative small portion included consideration of roots. The most frequently examined root response to elevated CO_2 were root dry weight, root length, root to shoot ratio and so on. Roots often exhibit the greatest relative dry weight increase among plant organs under high CO_2 (Wittwer 1978; Rogers *et al.* 1983; Imai *et al.* 1985; Norby *et al.* 1992). In this study, $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 resulted in more belowground dry weight, longer root. The primary yield components of the plant are produced by aboveground part (Idso *et al.* 1988). The substances needed for root growth and metabolism derived from leaves. From this point, the general conclusion can be drawn that the ratio of root dry mass to shoot dry mass is essential to analyze the change of carbon allocation patterns under elevated CO_2 concentration. From a subset of 224 observations, the log-transformed mean response of root-to-shoot ratio was a 6 percent increase for plants grown under elevated CO_2 . In our study, root-to-shoot ratio under $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 was 0.324 whereas 0.314 in the control chamber and 0.260 on the open site. But it was 0.246 under $500 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 . The ratio in-

creased by 3.1% and 19.8% under $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 compared with control and open site respectively. The seedlings grown at $500 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 had a more aboveground biomass and less belowground dry weight, which resulted in the lowest root-to-shoot ratio.

This paper showed that Korean pine seedlings grown under $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 allocated proportionately more of carbon belowground, causing an increase in root-to-shoot ratio. Bazzaz also accepted that there generally is an increase in allocation to roots (Bazzaz 1990). The belowground dry weight was enhanced by 44.8% comparing $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 with open site whereas taproot length and diameter increased by less than 10%. While these increased soluble sugar concentrations are not necessarily indicative of increased photosynthate allocation to roots, they may be important as carbon reserves (Chomba *et al.* 1993). Korean pine seedlings grown at $500 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 concentration showed lower root growth than ambient CO_2 except 32.2% increase of root dry weight compared with open site. Although $500 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 improve the aboveground growth, root systems were slightly affected. Maybe this result isn't beneficial to whole plant. This is because root systems can provide critical feedback on plant growth (Richard 1994) by altering the capacity of the plant to acquire sufficient water and nutrition from the environment. Elevated CO_2 increased total dry weight of plants. It must be pointed out that there was opposite response to the $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 between photosynthesis and biomass. Photosynthesis is an instant reaction whereas biomass is the accumulation course. So, the research of Korean pine seedlings' response to $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 needs making further. On the whole, there is a different response to slightly increased CO_2 and doubled CO_2 for Korean pine seedlings.

The Open Research Station of Changbai Mountain Forest Ecosystems is the important international long-term ecological research (LTER). The study mimicking the influence of the CO_2 -enriched air on Korean pine seedlings may have critical ecological implications. It provides a valuable result of the influence of different high CO_2 on aboveground and belowground physiological reaction and carbon distribution. The CO_2 concentration in atmosphere will increase gradually and slow. The upward regulation under $500 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 and downward regulation under $700 \mu\text{L}\cdot\text{L}^{-1}$ CO_2 of photosynthetic capacity in Korean pine seedlings in this study indicated that it would adapt to high CO_2 in the future. Korean pine seedlings may have potential acclimation ability to high CO_2 . Our study provides proper perspective to predict trees responses to elevated CO_2 as well as global climate change and carbon balance of whole ecosystem.

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